REMARKS

Reconsideration of the allowability of the present application is respectfully requested.

Status of the Claims

Claims 54-56, 59-92, and 95-142 have been acted upon by the Examiner. No claims have been allowed. Claims 54, 76, 79, 82, 83, 86, 87, 90, 111, 116, 118, 120, 122, 124, 126, 128, 130, 132 and 134-142 have been amended. No claims have been canceled. Claims 143-151 have been added. Accordingly, Claims 54-56, 59-92, and 95-151 are presented for examination

Claims 54, 76, 79, 82, 83, 86, 87, 90, 111, and 134-142 have been amended to add the recitation "wherein the albumin solution is a recombinant albumin solution." Support for this amendment can be found on page 1, lines 3-6, and page 25, lines 10-11.

Claims 86, 87, 139 and 140 have also been amended for clarity.

Claims 116, 118, 120, 122, 124, 126, 128, 130, 132 and 134-142 have been amended to change the range of albumin concentration subjected to the negative mode of cation exchange chromatography step from 20-70 g/L to 20-250 g/L. Support for this amendment can be found in original Claim 25, and on page 6, lines 19-21 of the application as filed.

Claims 134-142 have also been amended to specify that the glycosylated albumin is enzymatically glycosylated. Support for this amendment can be found on page 26, lines 20-22 of the application as filed.

Claims 143-151 have been added to recite a concentration step prior to the negative cation exchange step. Support for these claims can be found in original Claim 27.

I. 35 U.S.C. § 102(b) Rejection

The Examiner has rejected Claims 54-56, 61, 63-65, 74-81, 90-92, 96, 98-100, 109-115, 134-136, 141 and 142 under 35 U.S.C. § 102(b) as being anticipated by Matsuoka *et al.* (EP 0 428 758) as evidenced by Cohn *et al.* (J. Am Chem. Soc., vol. 68, pp. 459-75, 1946), Shaklai *et al.* (J. Biol. Chem., vol. 259, pp. 3812-17) and Ohmura *et al.* (EP 0 570 916 A2).

The Examiner asserts that Matsuoka *et al.* teaches a process for purifying album comprising a negative cation exchange (CE) chromatography step, an anion exchange (AE) chromatography step, and the placing of the purification product into a final container for therapeutic use. As Matsuoka *et al.* discloses the use of a Cohn V fraction, the Examiner relies on Cohn *et al.* for the proposition that the negative CE column of Matsuoka *et al.* is loaded with about 80 g/L of albumin. The Examiner relies on Shaklai *et al.* for the proposition that 10% of albumin present in human plasma is glycosylated, and because Matsuoka *et al.* teaches using a negative CE step, Matsuoka *et al.* inherently teaches binding of the glycosylated albumin to the CE resin. The Examiner further relies on Ohmura *et al.* for the proposition that the SP-Sepadex column disclosed in Matsuoka *et al.* refers to suplfopropyl-dextran.

Independent Claims 54, 76, 79, 90, 111, 134-136, 141 and 142 have been amended to add the recitation "wherein the albumin solution is a recombinant albumin solution." Each of the remaining rejected claims depends directly or indirectly from one of the amended independent claims.

A. Discussion of Matsuoka *et al.* (as evidenced by Cohn *et al.* and Shakalai *et al.*)

Masuoka *et al.* discloses a "process for producing an albumin preparation by treating an aqueous solution containing *serum* albumin first with an anion exchanger and second with a cation exchanger and followed by heat treatment." Page 2, lines 36-38 (emphasis added). Accordingly, Masuoka *et al.* is concerned with removing serum-derived contaminants.

As an example of the starting material, Matsuoka *et al.* discloses the use of "fraction V obtained by Cohn's cold alcoholic fractionation." (Page 2, lines 44-45). Cohn *et al.* discloses a system for the fractionation of human blood (serum). Because the Cohn V fraction was obtained from serum, it will contain albumin and serum-derived contaminants. In fact, Cohn *et al.* identifies the content of fraction V paste as containing 95% albumin, 4% α-globulin and 1% β-globulin. (Page 474, Table IX). Moreover, Matsuoka *et al* also reports that serum-derived albumin preparations may contain transferrin (Tf) which is difficult to remove due to its similar physiochemical property to albumin (page 2, lines 23-27) and

shows that Cohn's fraction V albumin contains transferrin so that, even after treatment with anion exchange chromatography, the sample still contains 8.3 mg/dl of transferrin (see Example 1 generally; page 5, lines 9-14 and Table 1 in particular). Additionally, Cohn *et al.* discloses that fraction V paste contains other, non-proteinaceous serum-derived contaminants, e.g. cholesterol and certain carbohydrates. (Page 474, Table VIII).

Shaklai *et al.* discloses that approximately 10% of the albumin in normal human serum is modified by nonenzymatic glycosylation, primarily at the ε-amino group of lysine residue 525. (See Abstract).

B. Claims As Amended Are Not Anticipated By Matsuoka *et al.*

The Examiner has rejected independent Claims 54, 76, 79, 90 and 111 over Matsuoka *et al.* (as evidenced by Cohn *et al.*) and independent Claims 134, 135, 136, 141 and 142 over Mastuoka *et al* (as evidenced by Cohn *et al.* and Shaklai *et al.*). In light of the above amendments, this objection is rendered moot. As amended, Claims 54, 76, 79, 90, 111, 134, 135, 136, 141, 142 and their dependent claims relate to a method performed on a *recombinant* albumin solution. Moreover, as amended, Claims 134, 135, 136, 141 and 142 additionally relate to a method performed on an albumin solution containing enzymatically glycosylated albumin.

1. Matsuoka *et al.* does not disclose a process for purifying a recombinant albumin solution

Matsuoka *et al.* discloses a process for purifying a serum-derived albumin solution; in particular, the starting material is identified as fraction V obtained by Cohn's cold alcoholic fractionation of serum. Whereas, applicants' claimed invention is directed to purifying a recombinant albumin solution. In contrast to the serum-derived contaminants discussed above, a recombinant albumin solution will comprise albumin and contaminants derived from the recombinant system used which, among other things, would not include α-globulin and β-globulin or the other plasma-derived contaminants discussed above.

Accordingly, it follows that a *recombinant* albumin solution will be physically distinct to a *serum-derived* albumin solution because the two solutions will contain *different types of contaminants*. Thus, a method that involves purifying a recombinant albumin solution is novel over a method that involves purifying a serum-derived albumin solution.

2. Matsukoa *et al.* does not disclose a process for removing enzymatically glycosylated albumin from unglycosylated albumin

In addition to the argument above, Claims 134, 135, 136, 141 and 142 are novel over Matsukoa *et al.* because, as amended the claims are directed to a process for purifying an albumin solution containing enzymatically glycosylated albumin.

Matsuoka *et al* discloses a method of purifying serum albumin. Serum albumin is not enzymatically glycosylated (see, e.g., page 26, lines 24-26 of the application). Shaklai *et al* states that 10% of albumin in normal human serum is modified by *nonenzymatic* glycosylation of serum albumin (emphasis added), primarily at the ε-amino group of lysine residue 525 (see abstract). Nonenzymatic glycosylation is also known as glycation (see application at page 64, first paragraph, first sentence). Shaklai *et al* does not suggest that serum albumin contains enzymatically glycosylated albumin.

Nonenzymatically glycosylated (i.e. glycated) albumin and enzymatically glycosylated albumin are physically different compounds.

Two types of enzymatic glycosylation exist: N-linked glycosylation to the amide nitrogen of asparagine and O-linked glycosylation to the hydroxy oxygen of serine and/or threonine. *See* Voet *et al.*, *Biochemistry*, John Wiley & Sons, Inc. 1990:570-71 (enclosed); Alberts *et al.*, *Molecular Biology of the Cell*, 1989:456 (enclosed). In contrast, as discussed above Shaklai *et al* discloses a nonezymatically glycosylated form of albumin that is glycated at the ε-amino group of lysine residue 525. Accordingly, enzymatically and nonenzymatically glycosylated forms of albumin are physically distinct.

Matsuoka *et al* does not disclose a method in which enzymatically glycosylated albumin is removed by cation exchange chromatography.

Accordingly, the claims of the present application as amended are clearly novel over Matsuoka *et al.*

C. Claims As Amended Are Non-Obvious Over Matsuoka *et al.*

The purification of albumin from a *recombinant* albumin solution using a method comprising the step of negative mode cation exchange ("CE") chromatography is also non-obvious over Matsuoka *et al.*

Matsuoka *et al* reports that its method of purifying albumin from serum is particularly concerned with the removal of *contaminating serum proteins* that are thought to cause albumin to become aggregated upon heat treatment, and also the removal of *serum-derived* proteins with similar physicochemical properties to albumin, such as transferrin (page 2, lines 18-27). The method of Matsuoka *et al* uses negative mode CE and AE steps, and Example 1 particularly shows that the negative mode CE is important in the avoidance of polymerization during heat treatment and the removal of transferrin.

However, the method claimed in the present application is directed to a different problem to that faced by Matsuoka *et al.*, namely to purify albumin from a *recombinant* albumin solution. As discussed above, a *recombinant* albumin solution will contain *different types and levels of contaminants*, compared to the *serum-derived* albumin solution that is treated in Matsuoka *et al.* If a skilled person wanted to purify albumin from a recombinant albumin solution, they would not have considered using the process steps of Matsuoka *et al.* The person skilled in the art would appreciate that the method steps of Matsuoka *et al* had been developed specifically to remove serum-derived contaminants, whereas a recombinant albumin solution would already be free of the human serum-derived proteins considered by Matsuoka *et al* to be problematic, i.e. those serum-derived proteins with similar physicochemical properties to albumin and the undefined human proteins that Matsuoka *et al* teach to contribute to albumin polymer formation during heat treatment.

In fact, none of the prior art documents disclose that a negative mode CE step can be useful in separating albumin from contaminants derived from a recombinant system. If the skilled person wished to purify albumin *from a recombinant albumin solution*, the key

teaching to follow is Goodey *et al* (WO 97/31947), which discloses a method of purifying recombinant albumin ("rHA") that relies on *positive mode* CE and AE steps.

In particular, Goodey *et al* teaches at page 6, lines 22-26:

> when the albumin is rHA produced in protease-deficient yeast or other organisms, the process does not normally comprise a heat treatment step....Similarly, if it is prepared from microorganisms (rather than from humans) it does not normally require a final pasteurisation step (typically 60 °C for one hour)

Therefore, as Goodey *et al.*'s method does not require rHA prepared from microorganisms to undergo heat treatment, even if the albumin were contaminated with serum-derived human proteins (which it would not be), the skilled person would not be motivated by Goodey *et al.* to remove such proteins by using the method disclosed by Matsuoka *et al.* because the lack of a heat treatment step removes the risk of polymerization.

In summary, the only teaching in the art relating to the purification of albumin from a *recombinant* albumin solution, using AE and CE steps, is Goodey *et al.*, and Goodey *et al.* is quite explicit in instructing its reader to use *positive mode* AE and CE steps. Thus, Goodey *et al.* teaches away from the methods claimed by the present invention, which relate to the use of CE in the *negative* mode. The reader of Goodey *et al.* would not be motivated by Matsuoka *et al* to use negative mode CE to purify albumin from a recombinant albumin solution because Matsuoka *et al* is concerned with the separation of albumin from different (serum-derived) contaminants, for different purposes. Accordingly, the claims are non-obvious over Matsuoka *et al.*

Moreover, Claims 134, 135, 136, 141 and 142 are non-obvious over Matsuoka *et al.* for the additional reason that there is no explicit suggestion in Matsuoka *et al* that its method would be suitable to remove any type of glycosylated albumin, let alone enzymatically glycosylated albumin. It is a surprising discovery by the present inventors that negative mode cation exchange chromatography can be used to remove enzymatically glycosylated albumin from unglycosylated albumin.

Accordingly, these claims are non-obvious in view of Matsuoka *et al* alone or in combination with any of the other cited publications.

II. 35 U.S.C. §103(a) Rejections

A. Claims 59-62 and 95-97

The Examiner has rejected Claims 59-62 and 95-97 under 35 U.S.C. § 103(a) as being unpatentable over Matsuoka *et al.* (as evidenced by Cohn *et al.*, Shaklai *et al.*, Ohmura *et al.*) and Goodey *et al.*(WO 97/31947). Claims 59-62 depend from independent Claim 54. Claims 95-97 depend from independent Claim 90.

The Examiner asserts that Matsuoka *et al.* teaches all of the limitations of Claims 59-62 and 95-97 other than the addition of an octanoate salt prior to the CE step. The Examiner further asserts that Goodey *et al.* teaches the addition of sodium octanoate in a final concentration of 1-10 mM and that it would have been obvious to have added the sodium octanoate of Goodey *et al.* to the albumin solution of Matsuoka *et al.* to protect albumin from polymerization as discussed in Goodey *et al.*

1. Discussion of Claimed Invention

Each of these claims has the feature that, prior to the cation exchange step, a fatty acid, such as an octanoate salt, is added to the albumin solution. Claims 59, 60, 95 and 96 specify particular concentrations of the octanoate ion to be present in the albumin solution subjected to cation exchange chromatography.

Moreover, this combination of subject matter provides an unexpected technical benefit. The present inventors found, surprisingly, that addition of octanoate to the albumin solution subjected to negative mode cation exchange chromatography results in the recovery of an increased yield of albumin, without affecting the level of contaminants.

The table below shows the result of an experiment in which solutions of impure albumin were prepared with different concentrations of octanoate and applied to a cation exchange chromatography column operated in the negative mode with respect to albumin. Albumin and contaminants were quantified in the combined flowthrough and wash fraction. In this connection, please see the declaration of inventor Philip Morton enclosed with this Reply which discusses this and other experiments.

Load octanoate concentration	**Albumin yield %**	**Detected contaminant level %**
4 moles.mole^{-1}	65	0.21
8 moles.mole^{-1}	73	0.23
12 moles.mole^{-1}	76	0.22

2. Discussion of Goodey *et al.*

Goodey *et al* teaches a method of albumin purification in which an albumin solution is applied to a cation exchanger. The albumin binds to the cation exchanger and is subsequently eluted from it (see page 21, lines 16-24). In other words, Goodey *et al* teaches a method of cation exchange chromatography that is run in the *positive mode* with respect to albumin. In contrast, it is apparent that the method of Matsuoka *et al* utilizes cation exchange chromatography in the *negative mode* with respect to albumin, as the albumin is said to be present in the pass-through and wash liquor (page 4, lines 56-58).

Goodey *et al* teaches in Example 2 on page 16 that octanoate may be added to condition the albumin prior to its application to the cation exchanger, and that the reason for doing so is to protect the albumin from polymerization. The skilled person, in light of Goodey *et al*, would therefore only consider including octanoate in the albumin solution during stages of the purification process in which albumin might be subject to polymerization.

3. Claims are non-obvious over Matsuoka *et al.* and Goodey *et al.*

Claims 59-62 and 95-97 depend from claims that as amended are directed to a process for purifying recombinant albumin. For all the reasons discussed above, purification of albumin from a *recombinant* albumin solution using a method comprising the step of negative mode cation exchange ("CE") chromatography is also non-obvious over Matsuoka *et al.* and Goodey *et al.*

It also would not have been obvious to the skilled person to modify the method of *negative mode* CE as disclosed in Matsuoka *et al* by adding fatty acid ions, such as octanoate ions, to the albumin as disclosed by Goodey *et al*, and thereby arrive at the method of Claims 59-62 and 95-97.

Polymerization of albumin molecules can only occur when individual albumin molecules come into close proximity, and it therefore follows that polymerization is more likely to occur at high concentrations of albumin than at lower concentrations. It is recognized in the art that polymerization of albumin is a particular problem at the very high concentrations of albumin present when albumin is precipitated or adsorbed to a solid phase. In this regard, Fisher *et al* (US Patent No. 4,228,154) is instructive. Fisher *et al.* teaches the following in the final paragraph of column 1:

> known ion exchange chromatographic methods for purification of albumin often involve multiple precipitation and resuspension steps...and further involve multiple desorptions of the albumin from the ion exchanger materials. Each such manipulation increases the potential for adverse changes in the native character of the albumin molecules.

The solution to the problem of albumin polymerization advanced by Fisher *et al* was the development of a method of purifying albumin in which "at no point in the process...is the albumin precipitated or removed from the solution phase" (column 2, lines 36-38).

The skilled person would not expect albumin polymerization to be problematic during negative mode cation exchange because, in such a procedure, the albumin always remains in solution. Accordingly, when an albumin solution is treated by negative mode cation exchange, it never attains the very high concentrations that can occur during a method that employs an albumin precipitation step, or the very high concentrations that occur when (as in Goodey *et al.*) a large number of albumin molecules are simultaneously immobilized in close proximity on an ion exchange matrix during positive mode ion exchange chromatography. As discussed above, Fisher *et al.* teaches that the use of negative mode ion exchange chromatography avoids adverse changes to albumin, e.g. *polymerization*, without suggesting the need for the addition of octanoate or other fatty acids.

Upon reading Matsuoka *et al.*'s negative mode cation exchange method, and being aware of Fisher *et al.*'s teaching that the use of the negative mode avoids changes in the native character of albumin, the skilled person would not consider that the albumin being applied to Matsuoka *et al.*'s method would be at risk of polymerization. Accordingly, the

skilled person would have had no reason to combine the teachings of Goodey *et al* and Matsuoka *et al* to arrive at the claimed invention.

None of the cited prior art discloses or suggests that including octanoate would have the advantageous effect of improving the albumin yield as discussed above, much less that this improvement could be achieved without compromising the purity of the product.

Accordingly, Claims 59-62 and 95-97 are non-obvious.

B. Claims 116-133

The Examiner has rejected Claims 116-121 and 130-133 under 35 U.S.C. §103(a) as being unpatentable over Matsuoka *et al.* (as evidenced by Cohn *et al.*, Shaklai *et al.* and Ohmura *et al.*).

The Examiner has rejected Claims 122, 123, 126 and 127 under 35 U.S.C. §103(a) as being unpatentable over Goodey *et al.*, Matsuoka-1 *et al.* (EP 0 428 758) (as evidenced by Cohn *et al.* and Shaklai *et al.*) and Matsuoka-2 *et al.* (U.S. Patent No. 5,277,818).

The Examiner has rejected Claims 124, 125, 128 and 129 under 35 U.S.C. §103(a) as being unpatentable over Goodey *et al.* and Matsuoka-1 *et al.* (as evidenced by Cohn *et al.* and Shaklai *et al.*), Matsuoka-2 *et al.*, and Chang.

Each of these rejections relies on the Examiner's assertion that Matsuoka *et al.* (Matsuoka-1) teaches subjecting the CE column to about 80 g/L, and that it would be obvious to one of skill in the art to optimize the conditions of Matsuoka *et al.* to fall within the claim recitation 20-70 g/L now amended to 20-250 g/L (Claims 116, 118, 120, 122, 124, 126, 128, 130 and 132) or 50 ± 10 g/L (Claims 117, 119, 121, 123, 125, 127, 129, 131 and 133)

1. Discussion of Claimed Invention.

Each of the rejected claims contains a recitation that the albumin solution subjected to the negative mode CE step has an albumin concentration of either 20-250 g/L or 50 ± 10 g/L.

In the previous Reply, applicants provided data to demonstrate that, unexpectedly following cation exchange chromatography, albumin yield is increased when albumin is applied to the column at a concentration that falls within the claimed concentration range of

20-250 g/L, and that contaminant levels are simultaneously decreased. Here we provide additional data to substantiate the effect at higher concentrations:

Load Concentration	Albumin yield %	Detected Contaminant Level %
5 $g.L^{-1}$	38	0.17
25 $g.L^{-1}$	66	0.12
50 $g.L^{-1}$	63	0.07
100 $g.L^{-1}$	75	0.09

Thus, at higher load concentrations, negative mode cation exchange chromatography provides for more efficient recovery of albumin (as the load concentration increases from 5 g/L to 100 g/L the albumin recovery increases from 38% to 75%). Moreover, at the same time, higher load concentrations result in impurities being more efficiently removed (contaminant levels reduced from 0.17% to 0.09%). These data substantiate that the inventive effect achieved using albumin at high concentrations occurs across the range claimed (20-250g/L). See Declaration of Philip Morton enclosed with this Reply.

2. Matsuoka *et al.* does not disclose negative mode CE chromatography using 80 g/L albumin.

Matsuoka *et al* does not explicitly disclose a suitable concentration range of albumin that may be subjected to the CE chromatography, but Example 1 describes how the method may be applied to an aqueous albumin solution derived from 500 g of a paste of fraction V obtained by Cohn's method of cold alcoholic fractionation. On page 5, first complete paragraph, the Examiner cited Cohn *et al* as evidence that fraction V contains about 96% albumin, and has then calculated that the solution applied to the CE chromatography column must have contained approximately 80 g/L albumin.

However, Cohn *et al* actually states, on page 471, fourth complete paragraph, that precipitate V is a paste containing about 250 g/L of protein, i.e. a 25% protein paste. If one were to take the density of Cohn precipitate V paste to be 1 g/ml (i.e. the same as water), then

500 g of fraction V paste (as used in Matsuoka *et al*) would have a volume of 500 ml, and 500 ml of a 25% protein paste would contain 125 g of protein.

It is likely that a 25% fraction V paste would be *more dense* than water (i.e. greater than 1 g/ml), in which case 500 g of paste would have a volume of less than 500 ml, and consequently a 25% protein paste of less than 500 ml volume would contain less than 125 g of protein. Accordingly, the 500g of Cohn's fraction V paste used in Example 1 of Matsuoka *et al* would contain, at most, 125 g of protein, *and probably less.*

Cohn *et al* indicates in Table IX on page 474 that 95% of the plasma protein of fraction V paste is albumin. Accordingly, it can be seen that Matsuoka's method used, at most, 118.75 g albumin (125g x 95%).

According to Matsuoka *et al* (see page 4, Example 1, parts 1-3), the 500g of fraction V paste was diluted in 2 L of water, then filtered, then further diluted by the addition of 2 L of water, and adjusted to pH 5.1, applied to the AE column and unbound albumin in the flow through collected and combined with 2 L of wash at pH 5.1 to take the total volume to 6 L. In other words, the albumin became diluted in a total of 6 L of solution. This would yield a solution of, at most, 19.8 g/L albumin. This assumes 100% recovery from the anion exchange step, although 100% is most unlikely and so in reality, it is probable that some albumin would clearly be lost in the anion exchange procedure.

In particular, the pH used by Matsuoka *et al* for the AE step would result in a substantial loss of albumin in this step. Full recovery of albumin from a negative mode AE step (as used in Matsuoka *et al*) relies on the albumin remaining neutral, or being positively charged, so as to avoid binding to the positively charged anion exchange matrix, whereas contaminant having a negative charge at the pH used will bind to the AE matrix and be separated from the albumin that is washed through. Accordingly, the operational pH of the AE step is key in determining whether any albumin becomes bound to the AE matrix and, as a result, is lost in the step. Matsuoka et al performs the AE step at pH 5.1 (page 4, lines 44-46), whereas albumin has an isoelectric point of 4.7 (as evidence see the enclosed extract from page 46 of "*All About Albumin: Biochemistry, Genetics and Medical Applications*" by Theodore Peters, Jr. Academic Press). Accordingly, Matsuoka et al performs the AE step at

a pH at which albumin will have a weak negative charge, and thus be able to bind to the AE matrix.

The effect of increasing the operational pH of the AE step on recovery (*i.e.* yield) of albumin from an albumin solution was investigated by the applicants, and increasing pH was shown to cause a reduction in yield from a negative mode AE step using DE-FF as the AE matrix:

Buffer Type	**Operational pH**	**Recovery**
20mM Sodium acetate equilibration buffer	4.5	98%
20mM Sodium acetate equilibration buffer	4.7	70%
40mM Sodium acetate equilibration buffer	4.5	96%
40mM Sodium acetate equilibration buffer	4.7	91%
40mM Sodium acetate equilibration buffer	4.9	81%

These data clearly show that increasing pH from 4.5 to 4.9 results in a substantial reduction in albumin recovery from a negative mode anion exchange step (up to 30% loss at pH 4.9 using the 20mM Sodium acetate equilibration buffer, which is the same buffer as used in Example 1 of Matsuoka *et al*). Accordingly, it is clear to see that using a pH of 5.1 (as in Matsuoka et al) would lead to even greater losses of albumin. See Declaration of Philip Morton enclosed with this Reply.

Accordingly, a maximum of 19.8 g/L albumin would be applied to the CE step of Example 1 of Matsuoka *et al*, and this figure does *not* account for the likelihood that (i) a 25% paste may be more dense than 1 g/L; and (ii) a substantial percentage of the total albumin will be lost in the anion exchange step, both of which would reduce the concentration of the albumin to under 19.8 g/L.

Furthermore, it is clear from a closer inspection of Matsuoka *et al* that the albumin is diluted in more than 6 L of solution. The pH was adjusted three times during the procedure, once with acetic acid, once with 1 N NaOH and once with 0.8 M sodium carbonate. Therefore, taking into account the diluting effect of these three steps, the albumin would clearly have been at a concentration of *less than 19.8 g/L* when applied to the CE column.

3. Claims are non-obvious over Matsuoka *et al.* alone or in combination with other publications

First, because the claims are directed to purifying a recombinant albumin solution, the claims are non-obvious for the reasons discussed in the section addressing the 35 U.S.C. § 102(b) rejection.

Second, the Examiner has not provided a *prima facie case* of obviousness. M.P.E.P. § 2144.05 II.B states that a particular parameter must first be recognized as a result-effective variable (i.e., a variable which achieves a recognized result) by the prior art, before the optimization of the variable can be characterized as routine experimentation. Here, none of the cited prior art discloses or suggests that albumin concentration is a key parameter in optimising recovery of the product and, contrary to the Examiner's allegation, it would therefore not have been obvious for the skilled person to examine the effect of increasing the albumin concentration when seeking to simultaneously improve the yield and purity of an albumin product. Accordingly, the Examiner's rejection is improper.

Third, the claimed range is non-obvious because the claimed range achieves unexpected results. M.P.E.P. § 2144.05 III ("Applicant can rebut a presumption of obviousness based on a claimed invention that falls within a prior range by showing . . . that there are new and unexpected results relative to the prior art."). At higher load concentrations, negative mode cation exchange chromatography provides for more efficient recovery of albumin (as the load concentration increases from 5 g/L to 100 g/L the albumin recovery increases from 38% to 75%). Moreover, at the same time, higher load concentrations result in impurities being more efficiently removed (contaminant levels reduced from 0.17% to 0.09%). See Declaration of Philip Morton attached to this Reply.

This result was contrary to expectations. In a negative mode cation exchange step, the impurities in the albumin are bound to the cation exchange matrix, whereas albumin passes through without binding. As the concentration of the solution applied to the cation exchange matrix is increased (i.e. as the concentrations of both the albumin and the impurities in the applied solution increases) one would have expected that the cation exchange matrix would

become less efficient due to increased demands being placed upon its capacity to bind and retain impurities. See Declaration of Philip Morton attached to this Reply.

Accordingly, Claims 116-133 are non-obvious over Matsuoka *et al.* alone or in combination with the other references cited by the Examiner.

C. Rejections Based on Goodey *et al.* and Matsuoka *et al.*

The Examiner has rejected Claims 54, 59-64, 66, 67, 69-71, 90, 95-99, 101, 102 and 104-106 under 35 U.S.C. §103(a) as being unpatentable over Goodey *et al.* and Matsuoka *et al.* (as evidenced by Cohen *et al.*, Shaklai *et al.* and Ohmura *et al.*).

The Examiner has rejected Claims 68, 72, 73, 103, 107 and 108 under 35 U.S.C. §103(a) as being unpatentable over Goodey *et al.* and Matsuoka *et al.* (as evidenced by Cohn *et al.*, Shaklai *et al.*, Ohmura *et al.*) as applied to Claims 67, 71, 102 and 106 above, and further in view of Ohmura *et al.* and Chang (EP 0 422 769 A1).

The Examiner has rejected Claims 82, 84, 86, 88, 137 and 139 under 35 U.S.C. § 103(a) as being unpatentable over Goodey *et al.*, Matsuoka-1 *et al.* (EP 0 428 758) (as evidenced by Cohn *et al.* and Shaklai *et al.*) and Matsuoka-2 *et al.* (U.S. Patent No. 5,277,818).

The Examiner has rejected Claims 83, 85, 87, 89, 138 and 140 under 35 U.S.C. §103(a) as being unpatentable over Goodey *et al.* and Matsuoka-1 *et al.* (as evidenced by Cohn *et al.* and Shaklai *et al.*), Matsuoka-2 *et al.*, and Chang.

In each of the above rejections, the Examiner relies on a combination of at least Goodey *et al.* and Matsuoka-1 *et al.* for the proposition that it would have been obvious to one of ordinary skill in the art to add the CE and AE steps run in the negative mode with respect to albumin of Matsuoka-1 *et al.* to the albumin purification method of Goodey *et al.* to meet the recitations of the rejected claims.

As discussed above, each of the claims contains the recitation or depends from a claim that contains the recitation "wherein the albumin solution is a recombinant albumin solution." Accordingly, as discussed above with respect to the § 102(b) rejection, the claims as amended are novel and non-obvious over Matsuoka-1 *et al.* or a combination of Matsuoka-

1 *et al.* or Goodey *et al.* as neither publication discloses purifying recombinant albumin by using a negative mode CE chromatography.

With respect to claims 137-140, these claims have been amended to recite "wherein the albumin solution subjected to cation exchange chromatography . . . contains enzymatically glycosylated albumin and the enzymatically glycosylated albumin is bound during the said cation exchange step." Accordingly, as discussed above with respect to the § 102(b) rejection, these claims as novel and non-obvious over Matsuoka-1 *et al.* or a combination of Matsuoka-1 *et al.* or Goodey *et al.* for the additional reason that neither publication discloses the removal of enzymatically glycosylated albumin from non-glycosylated albumin.

D. New Claims 143-151

Claims 143-151 have been added to recite a concentration step prior to the negative cation exchange step. Claims 143-151 depend from Claims 116, 118, 120, 122, 124, 126, 128, 130 and 132, respectively. Support for these claims can be found in original Claim 27. New Claims 143-151 are non-obvious over Mastuoka-1 *et al.*

As discussed above, Matsuoka-1 *et al* does not disclose a method in which albumin is applied to a cation exchange column at a concentration of 20-250 g/L. On the contrary, Matsuoka-1 uses a *lower* concentration.

Furthermore, in Matsuoka-1 *et al*, there is no appreciation that the concentration of albumin at this step is a parameter that should be controlled in order to achieve a simultaneous improvement in albumin yield and purity. Example 1 of Matsuoka-1 *et al* describes how a Cohn fraction V paste is dissolved to make an albumin solution, and how that solution is *diluted*, then applied to an anion exchanger and *further diluted* when the albumin in the flow through is combined with the wash liquor. The step of washing the anion exchanger is clearly intended to maximise the recovery of albumin; that the albumin solution becomes further diluted does not define the purpose of the step, but is merely a consequence. The albumin solution applied, by Matsuoka-1 *et al*, to the cation exchanger can be seen to arise through *a series of dilution steps*. The final volume of solution that the albumin is dissolved in is not chosen such that the albumin concentration falls within a particular range,

but is a consequence of the various dissolving, dilution, pH adjustment and washing steps that the albumin solution is subjected to.

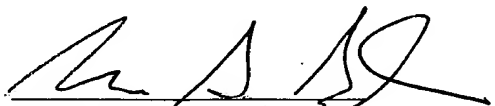
In contrast, the present claims specify that the albumin is conditioned by the deliberate step of *concentration* prior to the cation exchange step. Concentration is an active process and its inclusion reflects that the present applicants have surprisingly demonstrated that it is beneficial to utilise a concentration of albumin of 20-250 g/L for application to the negative mode cation exchange step in order to improve the albumin yield and purity in the product of the CE step.

Accordingly, the claims are novel and non-obvious in view of Matsuoka-1 *et al*, alone or in combination with any of the cited publications.

III. Conclusion

In view of the proposed claim amendments and the arguments presented above, the present application is believed to be in condition for allowance and an early notice thereof is solicited. The Office is invited to contact the undersigned counsel in order to further the prosecution of this application in any way.

Respectfully submitted,



Marc S. Segal, Esquire
Registration No. 40,163

Synnestvedt & Lechner LLP
2600 Aramark Tower
1101 Market Street
Philadelphia, PA 19107-2950
Telephone - (215) 923-4466
Facsimile - (215) 923-2189